NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 391

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TRIS(2-CHLOROETHYL) PHOSPHATE

(CAS NO. 115-96-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 8

PEER REVIEW PANEL 9

SUMMARY OF PEER REVIEW COMMENTS 10

INTRODUCTION 13

MATERIALS AND METHODS 17

RESULTS 25

DISCUSSION AND CONCLUSIONS 51

REFERENCES 55

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Gavage Study of Tris(2-Chloroethyl) Phosphate 59

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Gavage Study of Tris(2-Chloroethyl) Phosphate 95

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Gavage Study of Tris(2-Chloroethyl) Phosphate 129

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Gavage Study of Tris(2-Chloroethyl) Phosphate 163

APPENDIX E Organ Weights and Organ-Weight-to-Body-Weight Ratios 193

APPENDIX F Genetic Toxicology 205

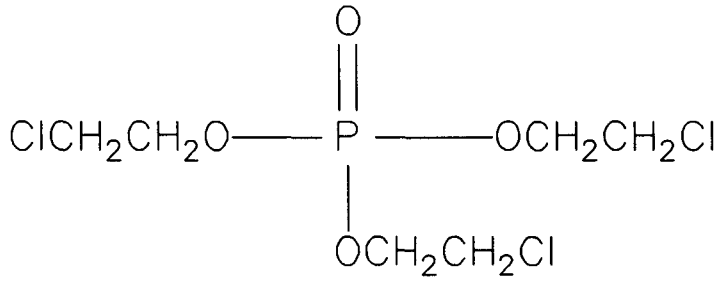
APPENDIX G Serum Cholinesterase Activity in the 16-Day and 16-Week Gavage Studies 213

APPENDIX H Chemical Characterization and Dose Formulation 217

APPENDIX I Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration 227

APPENDIX J Sentinel Animal Program 231

ABSTRACT



TRIS(2-CHLOROETHYL) PHOSPHATE

CAS No. 115-96-8
$C_6H_{12}Cl_3PO_4$
Molecular Weight: 285.5

Synonyms: 2-Chloroethanol phosphate (3:1), Tris(β-chloroethyl) phosphate
Trade Names: Fyrol CEF, Disflamoll TCA, NIAX flame retardant

Tris(2-chloroethyl) phosphate (TRCP), a flame-retardant plasticizer used in plastics, polymeric foams, and synthetic fibers, was studied as part of the National Toxicology Program's class study of trisalkyl phosphate flame retardants. Toxicology and carcinogenesis studies were conducted by administering TRCP (approximately 98% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 16 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

16-Day Studies: There were no chemical-related deaths, differences in final mean body weight, or histopathological lesions in rats receiving 22 to 350 mg/kg TRCP or in mice receiving 44 to 700 mg/kg TRCP for 12 doses over 16 days. Serum cholinesterase activity in female rats receiving 175 or 350 mg/kg TRCP was reduced slightly (80% of control levels), but enzyme activity in dosed male rats and in mice was similar to that in controls.

16-Week Studies: Rats received 22 to 350 mg/kg TRCP for 16 weeks (female) or 18 weeks (male). Several male and female rats in the 175 or 350 mg/kg dose groups died from chemical toxicity. Final mean body weights of female rats receiving 350 mg/kg were 20% greater than those of controls; final mean body weights of the remaining groups of dosed female rats and dosed male rats were similar. Chemical-related neuronal necrosis occurred in the hippocampus and thalamus of female rats and, to a lesser extent, of male rats. Serum cholinesterase activity was reduced in females receiving 175 or 350 mg/kg TRCP.

There were no chemical-related deaths, differences in final mean body weight, or differences in cholinesterase activity in mice receiving 44 to 700 mg/kg TRCP for 16 weeks. Tubule epithelial cells with enlarged nuclei (cytomegaly and karyomegaly) were observed in the kidneys of high-dose (700 mg/kg) male and female mice.

2-Year Studies: The 2-year studies in rats were conducted by administering 0, 44, or 88 mg/kg TRCP to groups of 60 males and females, 5 days per week for up to 104 weeks; 9 or 10 rats of each dose group were evaluated at 66 weeks. The survival of high-dose male and female rats was reduced relative to that of controls. Final mean body weights of surviving rats were similar to those of controls. The principal chemical-related effects occurred in the kidney and brain of dosed rats. Focal hyperplasia of the renal tubule epithelium and renal tubule adenomas were markedly increased in male rats receiving 88 mg/kg TRCP and, to a lesser extent, in female rats (renal tubule hyperplasia, male rats: 0/50; 2/50; 24/50; female rats: 0/50; 3/50; 16/50; renal tubule adenoma, male rats: 1/50; 5/50; 24/50; female rats: 0/50; 2/50; 5/50). Renal tubule carcinomas occurred in one control and one high-dose male rat. Degenerative lesions consisting of gliosis, mineralization, hemorrhage, and/or hemosiderin accumulation occurred in the cerebrum and brain stem of more than 50% of female rats receiving 44 or 88 mg/kg TRCP; similar lesions were seen in only a few dosed males. Slightly increased incidences of thyroid gland follicular cell neoplasms (male rats: 1/50; 2/48; 5/50; female rats: 0/50; 3/50; 4/50) and mononuclear cell leukemia (male rats: 5/50; 14/50; 13/50; female rats: 14/50; 16/50; 20/50) occurred in dosed males and females, but it is uncertain whether these were related to chemical administration.

The 2-year studies in mice were conducted by administering 0, 175, or 350 mg/kg TRCP to groups of 60 males and females, 5 days per week for up to 104 weeks; 8 to 10 mice of each sex per dose group were evaluated at 66 weeks. There were no significant differences in survival between dosed and control groups of either sex, and final mean body weights of mice were similar among all groups. The principal chemical-related effects occurred in the kidney, in which nuclear enlargement (karyomegaly) of tubule epithelial cells was present in approximately 80% of high-dose mice. In the original diagnosis, renal tubule adenomas were seen in one control male, one high-dose male, and one low-dose female. A carcinoma was also seen in one high-dose male. In a subsequent examination of step sections of all the mouse kidneys, adenomas were found in one low-dose male and two high-dose males. The incidences of renal tubule neoplasms in the original and step sections combined were 1/50, 1/50, and 4/50 for males. Female mice receiving TRCP demonstrated a marginally increased incidence of neoplasms (primarily adenomas) of the harderian gland (3/50; 8/50; 7/50); in addition, three harderian gland neoplasms occurred in high-dose female mice evaluated after 66 weeks.

Genetic Toxicology: TRCP was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without exogenous metabolic activation (S9), and it tested negative for the induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells. TRCP produced an equivocal response in the presence of S9 for the induction of sister chromatid exchanges (SCE) in CHO cells.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** for male and female F344/N rats receiving tris(2-chloroethyl) phosphate as shown by increased incidences of renal tubule adenomas. Thyroid follicular cell neoplasms and mononuclear cell leukemia in male and female rats may have been related to chemical administration. There was *equivocal evidence of carcinogenic activity* for male $B6C3F_1$ mice as shown by a marginally increased incidence of renal tubule cell neoplasms. There was *equivocal evidence of carcinogenic activity* for female $B6C3F_1$ mice as shown by a marginally increased incidence of harderian gland adenomas.

Renal tubule cell hyperplasia in male and female rats and gliosis, hemorrhage, pigmentation (hemosiderin accumulation), and mineralization in the brains of female rats were associated with the administration of tris(2-chloroethyl) phosphate. Karyomegaly of renal tubule epithelial cells in male and female mice was also chemical related.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tris(2-Chloroethyl) Phosphate

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 44, or 88 mg/kg 5 days per week	0, 44, or 88 mg/kg 5 days per week	0, 175, or 350 mg/kg 5 days per week	0, 175, or 350 mg/kg 5 days per week
Body weights	Dosed similar to controls	Dosed similar to controls	Dosed similar to controls	Dosed similar to controls
2-Year survival rates	36/50, 33/50, 25/50	32/50, 33/50, 17/50	25/50, 25/50, 25/50	31/50, 37/50, 35/50
Nonneoplastic effects	Renal tubule hyperplasia	Renal tubule hyperplasia; gliosis, hemorrhage, hemosiderosis, and mineralization in the cerebrum and brain stem	Karyomegaly of renal tubule epithelial cells	Karyomegaly of renal tubule epithelial cells
Neoplastic effects				
Chemical-related effects	Renal tubule adenomas (1/50; 5/50; 24/50); renal tubule carcinoma (1/50; 0/50; 1/50)	Renal tubule adenomas (0/50; 2/50; 5/50)	None attributed to TRCP	None attributed to TRCP
Equivocal effects	Thyroid follicular cell neoplasms; (1/50; 2/48; 5/50) mononuclear cell leukemia (5/50; 14/50; 13/50)	Thyroid follicular cell neoplasms; (0/50; 3/50; 4/50) mononuclear cell leukemia (14/50; 16/50; 20/50)	Renal tubule neoplasms (adenoma: 1/50; 1/50; 3/50; carcinoma: 0/50; 0/50; 1/50)	Harderian gland (adenoma or carcinoma: 3/50; 8/50; 7/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Equivocal evidence	Equivocal evidence
Genetic toxicology				
Salmonella typhimurium				
Gene mutation:	Negative with and without S9			
Sister chromatid exchanges				
Chinese hamster ovary cells *in vitro*:	Negative without S9; equivocal with S9			
Chromosomal aberrations				
Chinese hamster overy cells *in vitro*:	Negative with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on tris(2-chloroethyl) phosphate on April 25, 1990, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS

On April 25, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of tris(2-chloroethyl) phosphate received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Health Sciences, Research Triangle Park, NC.

Dr. H. B. Matthews, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (*clear evidence of carcinogenic activity* for male and female rats, *no evidence of carcinogenic activity* for male mice, and *equivocal evidence of carcinogenic activity* for female mice).

Dr. Matthews reported on step sectioning of the kidneys in mice, which revealed in males an additional control mouse with hyperplasia, an adenoma in one mouse in the low-dose group, and two additional hyperplasias and two adenomas in the high-dose group. Because of the additional kidney tumors found in dosed male mice, Dr. Matthews said that consideration should be given to changing the conclusion to *equivocal evidence.*

Dr. Garman, a principal reviewer, was in general agreement with the conclusions. However, he was not convinced that the increased incidence of harderian gland lesions in female mice was related to chemical treatment. And, with the additional information from the step sections of the kidneys in mice, he thought the level of evidence for male mice might be raised.

Dr. Gold, the second principal reviewer, agreed with the conclusions in male rats and male and female mice. She thought that the evaluation in male rats should be based on combined renal tubule carcinomas and adenomas, rather than on the adenomas alone. In female rats, she thought the low incidence of benign kidney tumors was supportive of only *some evidence of carcinogenic activity.* Dr. Gold questioned whether the incidence of a rarely observed tumor, granular cell tumors of the brain, in male and female rats might support equivocal evidence. Dr. S. Eustis, NIEHS, commented that these tumors are of meningeal origin in the rat and the meninges are rarely a site of carcinogenic activity even with a potent carcinogen, and thus, these tumors were not considered chemically related. Dr. Silbergeld was unconvinced that there was not a relationship, particularly since the site and mode of neurotoxic action apparently had not been characterized for this chemical. Dr. Matthews stated that there had been neurotoxicity studies done including some brain chemistry as well as evaluation of delayed behavioral effects after a single dose.

Dr. McKnight, the third principal reviewer, agreed with the overall conclusions for rats and mice. However, for mice, she thought that, based on survival rates and weight gain in the 2-year studies, it was not clear that the maximum tolerated dose was achieved. Dr. Matthews said that the significantly increased incidences of renal tubule karyomegaly at low and high doses in both sexes indicated that adequate doses had been used. For female rats, Dr. McKnight noted that the significant positive trend for thyroid follicular cell neoplasms and the significantly greater incidence in high dose versus control supported including them under *clear evidence.* Dr. Eustis responded that the small numbers of tumors and the absence of increases in preneoplastic lesions (hyperplasias) spoke against raising the level of evidence. For male rats, Dr. McKnight argued that mononuclear cell leukemias should be included under *clear evidence* based on a significant positive trend test and positive pairwise comparisons for both high- and low-dose groups with controls. Dr. McKnight thought too much emphasis was put on the highly variable historical control range as contrasted to the concurrent control values for leukemias in discounting their significance in the TRCP studies. Dr. J. Hasman, NIEHS, agreed that the primary emphasis should be on concurrent controls, but felt that it was also important to consider that the leukemia rate in high-dose male rats was essentially identical to the average control response for the three previous studies in the same laboratory.

Dr. Garman moved that the conclusions be accepted as written for male and female rats, *clear evidence of carcinogenic activity.* Dr. Longnecker seconded the motion, which was accepted by seven "yes" votes (Drs. Carlson, Davis, Hayden, Longnecker, McKnight, Silbergeld, Zeise) to four "no" votes

(Drs. Ashby, Garman, Gold, Goodman). Dr. Garman moved that the conclusions for male mice be changed from *no evidence of carcinogenic activity* to *equivocal evidence of carcinogenic activity* based on the additional renal tubule neoplasms revealed in the resectioning examination. Dr. Ashby seconded the motion, which was accepted unanimously with 11 votes. Dr. Garman moved that the conclusions be accepted as written for female mice, *equivocal evidence of carcinogenic activity.* Dr. Ashby seconded the motion, which was accepted unanimously with 11 votes.